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Protective effect of aqueous extract of Lophira *lanceolata* leaf against cisplatin-induced hepatorenal injuries and dyslipidemia in Wistar rats

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Abstract

Background: Hepatorenal injuries and dyslipidemia are common global health challenges but medicinal plant extracts may have potential to prevent them. Thus, this study evaluated the protective effect of aqueous extract of *Lophira lanceolata* leaf (LLE) against cisplatin-induced hepatorenal injuries and dyslipidemia in albino Wistar rats.

Methods: Thirty rats were randomly divided into 6 groups of 5 rats each. Group I rats received distilled water and served as control, group II rats were given 5 mg/kg cisplatin (CIS) intraperitoneally, groups III and IV rats were treated with 200 and 400 mg/kg LLE respectively for 26 days by oral gavages while groups V and VI rats were treated with 200 and 400 mg/kg LLE respectively, followed by CIS on the 21st day as in group II. About 24 h after treatment, blood was collected from the rats; then serum was separated and used for estimations of biochemical parameters. The kidney and liver of rats were removed, rinsed in normal saline, stored in 10% formalin and used for histological analyses.

Results: The biomarkers of hepatic (Aminotransferases, Alkaline phosphatase and Bilirubin) and renal (urea and creatinine) injuries, and dyslipidemia (Total cholesterol, triglycerides and LDL-cholesterol) significantly ($p < 0.05$) increased in the rats exclusively exposed to cisplatin when compared with normal control. However, treatment of cisplatin-exposed rats with 200 and 400 mg/kg LLE significantly ($p < 0.05$) reduced the levels of these biomarkers of hepatorenal injuries and dyslipidemia when compared with cisplatin control. Photomicrographs showed pathological signs in the liver and kidney of rats exclusively exposed to cisplatin, but there was moderate protection of these tissues in the rats treated with LLE and cisplatin.

Conclusion: The current findings have shown that *Lophira lanceolata* leaf extract may provide moderate protection against cisplatin-induced hepatorenal injuries and dyslipidemia in albino Wistar rats.

Keywords: Hepatotoxicity, Histopathology, Hyperlipidemia, Nephrotoxicity

Introduction

The liver plays a central role in transforming and detoxifying chemicals, so it is susceptible to their toxic effects [1]. Drug-induced liver injury is a cause of acute and chronic liver diseases in animals and humans. Recent epidemiological data suggests that approximately 20 new cases of drug-induced liver injury (DILI) per 100,000 persons occur annually [2]. The Kidney plays a role in homeostasis and excretion of drugs, so it is exposed to drug-induced injuries [3]. The incidence of drug-induced kidney injury has increased due to increasing use of drugs, as they are readily available as over-the-counter medicines, especially the non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics [4]. About 20% of nephrotoxicity is induced by drugs but medication of the elderly increases this incidence up to 60% as the average lifespan increases [5]. Dyslipidemia is a condition characterized by alteration in the plasma concentrations of lipids (triglycerides and total cholesterols)

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and the blood transporting lipoproteins (LDL-cholesterol, HDL-cholesterol and VLDL-cholesterol). Hyperlipidemia is a common form of dyslipidemia in animals [6].

Cisplatin, chemically known as cis-diamine dichloroplatinum II (CDDP), with chemical formula cis-[Pt $(NH_3)_2(Cl)_2$], is a platinum-based chemotherapeutic agent used for treatment of various types of cancers [7]. It is associated with several toxic side effects including nephrotoxicity, hepatotoxicity and oxidative stress injuries. The adverse effects of cisplatin occur by increased production of reactive oxygen species (ROS) and decreased production of antioxidants, though the formation of ROS depends on the concentration and duration of exposure to the drug [8].

Herbal medicine is increasingly getting attention as a useful tool in the management of several disorders. *Lophira lanceolata* known as "Iron wood" (English) is a tree that grows up to 12 m high, with a narrow crown, deep root, less scaly bark and contains no latex [9]. It belongs to the Family: Ochnaceae, widely distributed in the West and Central African countries including Nigeria and Cote d'Ivoire [10]. In Nigeria, it is called "Okopia" (Igbo), "Ikponhon" (Yoruba), "Okopi" (Idoma) and "Namijin kadanya" (Hausa) [11]. It is used for the treatment of dermatosis, toothache and muscular tiredness [9]. It is used by many communities in northern Nigeria for the treatment of epilepsy and erectile dysfunction [10]. Previous scientific studies on the leaf extract of this plant revealed antiplasmodial [12] and antibacterial [13] activities. However, there may be paucity of scientific report on the effect of *L. lanceolata* leaf extract on drug-induced hepatorenal disorders and dyslipidemia in animals. Thus, this study was initiated to investigate the protective effect of aqueous extract of *L. lanceolata* leaf against cisplatin-induced hepatorenal injuries and dyslipidemia in albino Wistar rats.

Materials and methods

Drug used for induction of hepatorenal injuries and dyslipidemia

Cisplatin was the drug of choice for induction of dyslipidemia, liver and kidney injuries in rats. It is an injectable liquid manufactured by Kwality Pharmaceuticals Pvt. Ltd., Nagkalan, Majitha Road, Amritsar, India.

Plant collection and preparation

The fresh leaves of *L. lanceolata* were collected from North Bank - Gbajimba road, in Makurdi, Benue State, Nigeria, and identified by a plant taxonomist. The plant leaves were air-dried at room temperature for 2 weeks, pulverized using a grinding machine, and sieved to obtain a fine powder. A 200 g of pulverized sample was macerated in 4000 ml of distilled water in a conical flask and covered. This was kept at room temperature for 48 h, with frequent agitation after every 2 h. The liquid mixture was filtered with Whatman no.1 filter papers. The filtrate was concentrated and dried by evaporation at 40 °C using a water-bath. The dried extract was weighed to determine the yield and stored in refrigerator until needed.

Experimental animals and management

Adult male albino Wistar rats (*Rattus norvegicus*) weighing 150–230 g, were purchased from the Animal house of College of Health Sciences, Benue State University, Makurdi, Nigeria. The rats were allowed to stabilize and acclimatize for at least 2 weeks before being used for the experiments. They were kept in plastic cages in well ventilated room, fed with standard animal feeds, produced by Grand Cereals and Oil Mills Ltd., Jos, and water *ad libitum*. The animals were handled with care, according to the principles and standard protocols for the use of laboratory animals for experiments.

Animal grouping and treatments

The rats were divided into six groups of five animals per group. Group I rats received only distilled water for 26 days by oral gavages and served as normal control. Group II rats were given distilled water for 26 days by oral gavages and administered 5 mg/kg body weight cisplatin by intraperitoneal (i.p.) route on the 21st day. Groups III and IV rats received 200 and 400 mg/kg b. wt. *Lophira lanceolata* extract (LLE) respectively for 26 days by oral gavages. Group V and VI rats received 200 and 400 mg/kg b. wt. LLE respectively for 26 days while cisplatin was administered on the 21st day as in group II. Body weights of the rats were recorded at the initial week, then second, third and last weeks of the experiment.

Collection and preparation of blood and tissues

About 24 h after treatments, rats were sacrificed under chloroform anaesthesia. Blood was collected from rats by intra-cardiac puncture and dispensed into plain sterilized tubes. The blood was allowed to stand on the bench for at least 2 h, spun with a centrifuge at 3000 rpm for 10 min, then serum was separated with clean Pasteur pipette and stored frozen until used for biochemical analyses. The liver and kidney of rats were excised, rinsed in normal saline, weighed and preserved in 10% formalin for histological analyses.

Biochemical assays

The biochemical parameters, which are markers of hepatorenal injuries and dyslipidemia, were estimated using assay kits according to the manufacturer's procedures. High density lipoprotein (HDL) cholesterol concentration was determined by the procedure described in the reagent kits instruction manual (AGAPPE Diagnostics

Ltd., Switzerland). Total cholesterol (TC) and triglyceride (TRG) concentrations were determined by the methods given in the reagent kits instruction manuals (TECO Diagnostics Ltd., USA). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by IFCC methods, using the kits instruction manual (AGAPPE Diagnostics Ltd). Alkaline phosphatase activity was determined by the method described in the assay kit instruction manual (TECO diagnostics Ltd., USA). Serum bilirubin levels were determined by the modified TAB method described in the assay kits manual (AGAPPE Diagnostic Ltd). The creatinine and total protein concentrations were determined by the methods described in assay kits instruction manuals (Randox Laboratories Ltd., UK). The urea and albumin concentrations were estimated by the methods described in assay kits manuals (AGAPPE Diagnostics Ltd).

The atherogenic/dyslipidemic indices were calculated using the equations, according to previously described methods [14, 15].

1. Cardiac risk ratio (CRR) = Total cholesterol/HDL cholesterol
2. Atherogenic coefficient (AC) = Total cholesterol − HDL cholesterol/HDL cholesterol
3. Atherogenic index of plasma (AIP) = Log (Triglycerides/HDL cholesterol)
4. Classical ratio (CR) = LDL cholesterol/HDL cholesterol

Histological analyses of liver and kidney tissues

Histopathological examination was carried out according to a previously described method [16]. The liver and kidney tissues were fixed in 10% formalin, dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin. The stained glass slides were viewed under light microscope at × 10 and 40 objective lenses.

Statistical analysis

The statistical package for the social sciences (SPSS version 21.0) produced by SPSS Inc., Chicago, USA, was used for statistical analysis. Data were presented as mean ± standard error of mean (SEM), with $n = 5$. The data were analyzed by one-way analysis of variance

(ANOVA), followed by least significant difference (LSD) as Post Hoc test for multiple comparisons. Mean differences were considered statistically significant at $p < 0.05$.

Results

Effect of *Lophira lanceolata* leaf extract on biomarkers of hepatic injuries in rats

There was a significant ($p < 0.05$) elevation in the activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin levels of cisplatin-exposed rats when compared with the normal control. There was no significant ($p > 0.05$) difference in the levels of total proteins and albumin of rats in all the treatment groups compared with normal control. However, treatment with aqueous extract of *L. lanceolata* leaf significantly ($p < 0.05$) reduced the levels of ALT, AST and total bilirubin of cisplatin-exposed rats when compared with the cisplatin control (Table 1).

Effect of *Lophira lanceolata* leaf extract on lipid profile and biomarkers of renal injuries in rats

There was a significant ($p < 0.05$) increase in the levels of T-cholesterol, triglycerides and LDL-cholesterol of cisplatin-exposed rats when compared with the normal control. There was no significant ($p > 0.05$) difference in the levels of HDL-cholesterol of rats in all the treatment groups. However, treatment with 200 and 400 mg/kg b. wt. aqueous extract of *L. lanceolata* leaf significantly ($p < 0.05$) reduced the levels of T-cholesterol, triglycerides and LDL-cholesterol of cisplatin-exposed rats when compared with the cisplatin control (Table 2). There was a significant ($p < 0.05$) increase in the levels of urea and creatinine of cisplatin-exposed rats when compared with normal control. However, treatment with 200 and 400 mg/kg b. wt. aqueous extract of *L. lanceolata* leaf significantly ($p < 0.05$) reduced the levels of urea and creatinine of cisplatin-exposed rats when compared with cisplatin control (Table 2).

Effect of *Lophira lanceolata* leaf extract on percentage organ to body weight ratio of rats

There was a significant ($p < 0.05$) reduction in the weights of liver, kidney, ratio of liver to body weight and kidney to body weight of cisplatin-exposed rats when compared with normal control. The treatment of cisplatin-exposed rats with 200 and 400 mg/kg LLE significantly ($p < 0.05$) increased their ratio of liver to body weight and kidney to body weight when compared with cisplatin control. There was also significant ($p < 0.05$) increase in the weights of liver and kidney of cisplatin-exposed rats treated with 200 mg/kg LLE when compared with cisplatin control. However, there was no significant ($p > 0.05$) difference in the weights of liver and kidney of rats given 200 and 400 mg/kg LLE only when compared with the normal control (Table 3).

Effect of *Lophira lanceolata* leaf extract on atherogenic/dyslipidemic indices in rats

There were increases in cardiac risk ratio (CRR), atherogenic coefficient (AC), classical ratio (CR) and atherogenic index of plasma (AIP) of cisplatin-exposed rats when compared with the normal control. The rats treated with 200 mg/kg b. wt. showed slight increases in

Table 1 Effect of *Lophira lanceolata* leaf extract on biomarkers of hepatic injuries in rats administered cisplatin

Treatment groups	Serum biomarkers of hepatic injuries					
	T-Protein (g/dL)	Albumin (g/dL)	ALT (U/L)	AST (U/L)	ALP (U/L)	T-Bilirubin (mg/dL)
I. Normal control	6.68 ± 0.69	3.00 ± 0.13	30.12 ± 3.46	27.56 ± 5.17	22.32 ± 6.59	0.62 ± 0.12
II. 5 mg/kg Cisplatin	6.42 ± 0.70	3.50 ± 0.32	178.0 ± 38.5[a]	73.44 ± 2.75[a]	48.04 ± 3.09[a]	3.78 ± 1.15[a]
III. 200 mg/kg LLE	6.48 ± 0.41	3.12 ± 0.26	33.72 ± 5.98[b]	37.14 ± 9.58[b]	35.32 ± 5.87	0.56 ± 0.09[b]
IV. 400 mg/kg LLE	5.56 ± 0.37	2.98 ± 0.16	31.50 ± 2.83[b]	30.22 ± 3.26[b]	39.36 ± 10.1	0.48 ± 0.17[b]
V. 200 mg/kg LLE + CIS	7.28 ± 0.88	3.12 ± 0.16	44.08 ± 8.18[b]	42.56 ± 4.49[b]	34.60 ± 6.28	0.40 ± 0.14[b]
VI. 400 mg/kg LLE + CIS	6.24 ± 0.42	2.90 ± 0.20	66.40 ± 6.65[b]	34.16 ± 2.82[b]	34.40 ± 5.36	1.86 ± 0.10[b]

Values are Mean ± SEM, $n = 5$; LLE - *Lophira lanceolata* leaf extract, CIS - Cisplatin
[a]significantly different from normal control ($p < 0.05$)
[b]significantly different from cisplatin control ($p < 0.05$)

CRR, AC, and CR while those treated with 400 mg/kg b. wt. showed slight decreases in CRR, AC and AIP when compared with normal control. However, the treatment of cisplatin-exposed rats with 200 and 400 mg/kg b. wt. LLE decreased their CRR, AC, CR and AIP when compared with cisplatin control. Though, the decreases in CRR, AC, CR and AIP values were greater by treatment of cisplatin-exposed rats with 200 mg/kg than 400 mg/kg LLE (Table 4).

Histopathological findings in liver and kidney of rats treated with *L. lanceolata* extract

The photomicrographs of liver and kidney sections of rat given only distilled water appeared normal, without signs of degenerative changes. The hepatocytes and liver sinusoids appeared normal while the glomerulus, Bowman's capsule and renal tubules also appeared normal (Figs. 1a and b). The photomicrographs of liver and kidney sections of a rat exposed to 5 mg/kg b.wt. CIS showed signs of hepatorenal injuries. There was sinusoidal dilatation, vascular congestion, infiltration of portal triad and stroma of liver by inflammatory cells, and foci of coagulative necrosis. The kidney tissues showed capsular dilation, distorted glomeruli, and congestion of interstitial spaces, showing signs of interstitial nephritis (Figs. 2a and b).

The photomicrographs of liver and kidney sections of rat treated with 200 mg/kg LLE showed mild hepatorenal injuries. There was mild congestion of interstitial spaces and foci of interstitial nephritis. There was infiltration of hepatic cords by few inflammatory cells and foci of mild coagulative necrosis (Figs. 3a and b). The photomicrographs of liver and kidney sections of rat treated with 400 mg/kg LLE showed mild hepatorenal injuries. There was mild congestion of renal interstitial spaces, and some glomeruli were shrunken with dilated capsular spaces. There was infiltration of portal triad and hepatic cords by inflammatory cells, and mild congestion of liver sinusoids (Figs. 4a and b).

The photomicrographs of liver and kidney sections of rat treated with 200 mg/kg LLE and exposed to 5 mg/kg CIS showed mild hepatorenal injuries. There were few shrunken glomeruli, infiltration of renal tubules by inflammatory cells and congestion of interstitial spaces, showing signs of mild interstitial nephritis. There was congestion of few sinusoids in the liver, which was mild when compared with the CIS control (Figs. 5a and b). The photomicrographs of liver and kidney sections of rat treated with 400 mg/kg LLE and exposed to 5 mg/kg CIS showed mild hepatorenal injuries and signs of recovery. There were pockets of hemorrhage seen in the

Table 2 Effect of *Lophira lanceolata* leaf extract on lipid profile and biomarkers of renal injuries in rats administered cisplatin

Treatment groups	Serum lipid profile and biomarkers of renal injuries					
	Total Chol. (mg/dL)	TRG (mg/dL)	HDL-Chol. (mg/dL)	LDL-Chol. (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
I. Normal control	116.6 ± 3.1	129.2 ± 5.1	36.82 ± 3.09	46.84 ± 3.15	27.46 ± 4.68	0.44 ± 0.17
II. 5 mg/kg Cisplatin	194.8 ± 24.0[a]	149.5 ± 2.9[a]	36.68 ± 3.20	133.5 ± 17.4[a]	65.14 ± 6.96[a]	1.92 ± 0.39[a]
III. 200 mg/kg LLE	127.2 ± 11.9[b]	117.7 ± 4.2[b]	37.00 ± 3.84	58.78 ± 8.20[b]	32.34 ± 3.71[b]	1.06 ± 0.24[b]
IV. 400 mg/kg LLE	94.2 ± 8.01[b]	136.1 ± 1.9	40.40 ± 1.30	56.22 ± 6.10[b]	33.08 ± 4.33[b]	0.48 ± 0.07[b]
V. CIS + 200 mg/kg LLE	109.3 ± 5.12[b]	146.6 ± 14.6	41.44 ± 2.24	31.72 ± 5.48[b]	30.28 ± 1.10[b]	0.46 ± 0.09[b]
VI. CIS + 400 mg/kg LLE	129.8 ± 0.82[b]	128.8 ± 1.9[b]	35.54 ± 4.73	80.92 ± 5.27[ab]	38.08 ± 5.67[b]	0.42 ± 0.19[b]

Values are Mean ± SEM, n = 5; LLE - *Lophira lanceolata* leaf extract, CIS - Cisplatin
[a]significantly different from normal control ($p < 0.05$)
[b]significantly different from cisplatin control ($p < 0.05$)

Table 3 Effect of *Lophira lanceolata* leaf extract on percentage of organ to body weight ratio of rats administered cisplatin

Treatment groups	Body weight and percentage of organ to body weight ratio				
	Body weights(g)	Liver weights(g)	Liver/Body weights (%)	Kidney weights(g)	Kidney/Body weights (%)
I. Normal control	195.0 ± 10.37	5.66 ± 0.28	2.9 ± 0.20	0.65 ± 0.05	0.33 ± 0.01
II. 5 mg CIS	233.8 ± 11.08[a]	4.25 ± 0.08[a]	1.8 ± 0.10[a]	0.49 ± 0.01[a]	0.21 ± 0.009[a]
III. 200 mg LLE	195.4 ± 5.27[b]	5.91 ± 0.09[b]	3.02 ± 0.07[b]	0.58 ± 0.01	0.30 ± 0.006[b]
IV. 400 mg LLE	198.6 ± 6.21	6.66 ± 0.27[b]	3.35 ± 0.16[b]	0.60 ± 0.01[b]	0.30 ± 0.008[b]
V. 200 mg LLE + CIS	209.0 ± 7.38	5.59 ± 0.38[b]	2.67 ± 0.14[b]	0.72 ± 0.01[b]	0.34 ± 0.01[b]
VI. 400 mg LLE + CIS	197.4 ± 3.78[b]	5.00 ± 0.27	2.53 ± 0.09[b]	0.76 ± 0.01[ab]	0.39 ± 0.02[b]

Values are Means ± SEM, n = 5; LLE - *Lophira lanceolata* leaf extract, CIS - Cisplatin
[a]significantly different from the normal control ($p < 0.05$)
[b]significantly different from the cisplatin control ($p < 0.05$)

interstitial spaces and signs of recovery from renal injuries. There was mild hemorrhage and infiltration of portal triad of the liver by inflammatory cells, which was less severe when compared with CIS control (Figs. 6a and b).

Discussion

The alterations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities are biochemical indicators of hepatocellular injuries [17]. The significant increase in serum AST and ALT activities, after exposure of rats to cisplatin may indicate liver injuries, attributed to the adverse effect of this drug. Previous studies have shown that increases in serum AST and ALT activities reflect hepatocellular membrane damage and leakage [2, 17]. The significant increase in alkaline phosphatase (ALP) activity may also indicate liver impairments and cholestasis, due to the adverse effect of cisplatin. ALP activity increases as a result of biliary obstructions or cholestasis, thus it is a biomarker of hepatobiliary disease [2].

The alteration in serum bilirubin levels is a biochemical indicator of the changes in morphological integrity of hepatobiliary tract, as a sign of liver damage [17–19]. Elevated levels of bilirubins may be due to excessive haemolysis or biliary tract obstruction [18]. The significant increase in serum total bilirubin may indicate impaired hepatic excretory function, a sign of liver dysfunction, attributed to the adverse effect of cisplatin. These findings are in agreement with El-Hosseiny et al. [2] who found significant elevation in the levels of total bilirubin, after exposure of rats to a drug. Bilirubin is the excretory product formed by catabolism of heme in hemoglobin, thus hyperbilirubinemia is usually caused by excessive destruction of heme and blockage of biliary tract; which results in severe inhibition of conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes [18]. The significant reduction of AST, ALT, ALP and bilirubin levels after treatment of cisplatin-exposed rats with LLE may indicate a protective effect of this plant extract against cisplatin-induced hepatic injuries. The LLE may have stabilized hepatocyte membrane integrity against cisplatin lethality, thereby preventing elevation of these biomarkers of hepatic injuries in rats. Some plant extracts have earlier been reported to produce protective effects against drug-induced liver injuries in animals by reduction of ALT, AST, ALP and bilirubin levels [2, 19].

Creatinine and urea levels are used as biochemical markers of kidney injuries, thus elevated levels of these markers may indicate kidney dysfunction, though the test for creatinine is a better indicator than urea [20]. Creatinine is a non-protein nitrogenous substance

Table 4 Effect of *Lophira lanceolata* leaf extract on atherogenic/dyslipidemic indices in rats administered cisplatin

Treatment groups	Atherogenic/dyslipidemic indices of rats			
	Cardiac risk ratio (CRR)	Atherogenic coefficient (AC)	Atherogenic index (AIP)	Classical ratio (CR)
Normal control	3.17	2.17	0.55	1.27
Cisplatin control	5.31 (67)[a]	4.31 (98)[a]	0.61 (11)[a]	3.64 (186)[a]
200 mg/kg LLE only	3.44 (9)[a]	2.44 (12)[a]	0.50 (9)[a]	1.59 (25)[a]
400 mg/kg LLE only	2.33 (27)[a]	1.33 (39)[a]	0.53 (4)[a]	1.39 (9)[a]
200 mg/kg LLE + CIS	2.64 (50)[b]	1.64 (62)[b]	0.55 (10)[b]	0.76 (79)[b]
400 mg/kg LLE + CIS	3.65 (31)[b]	2.65 (39)[b]	0.56 (8)[b]	2.27 (38)[b]

Values are means of 5 rats; Values in parenthesis are percentage changes (%), CIS - Cisplatin, LLE - *Lophira lanceolata* leaf extract
[a]percentage changes when compared with normal control
[b]percentage decrease when compared with cisplatin control

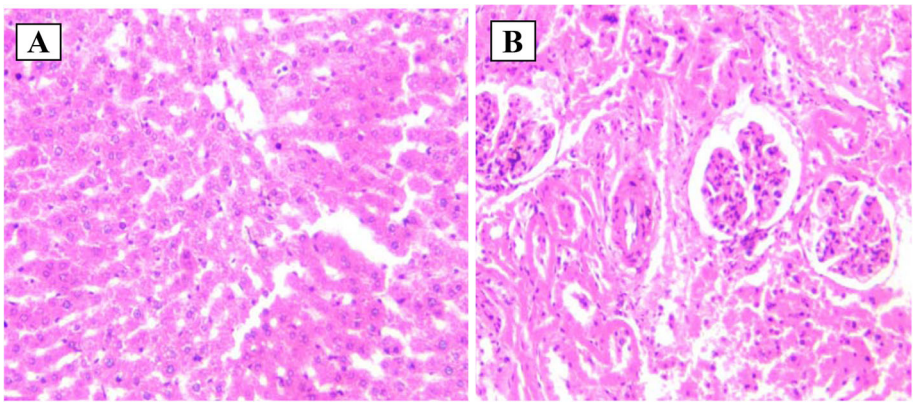

Fig. 1 Photomicrographs of liver and kidney of rat given distilled water only (× 100, H & E stain); Morphology of liver tissue (**a**) showing normal hepatocytes, without any change in the tissue structural architecture, and kidney tissue (**b**) with normal glomeruli, Bowman's capsules and renal tubules

formed from creatine and phosphocreatine during muscle metabolism but excreted by glomerular filtration. Just like urea, the rate of excretion of creatinine is influenced by glomerular filtration rate (GFR), so any abnormality that decreases GFR will result in an increased serum creatinine [21, 22]. The significant increase in urea and creatinine levels after exposure of rats to cisplatin may be an indication of kidney impairments, due to the adverse effect of this drug. These findings suggest that there may be reduction in GFR of the animals and are in agreement with the findings of Kim et al. [1] and Rajakrishnan et al. [20] who reported that the alterations induced in kidneys by cisplatin were characterized by elevated levels of their injury markers. The significant reduction of urea and creatinine levels after treatment of rats with LLE and cisplatin may suggest that this plant extract has protective effect against cisplatin-induced kidney injuries in animals. The capacity of this plant extract to decrease urea and creatinine levels in rats may be attributed to its ability to protect the nephrons and increase GFR, as a result of its phytochemicals [21]. Previous studies have shown that certain plant extracts have protective effects against cisplatin-induced kidney injuries in animals by reduction of urea and creatinine levels [1, 20, 22].

The liver plays a major role in controlling plasma levels of cholesterol, thus when there is drug-induced liver impairments, there will be elevated levels of serum total cholesterol (TC) and LDL-cholesterol [17]. The significant increase in serum levels of total cholesterol

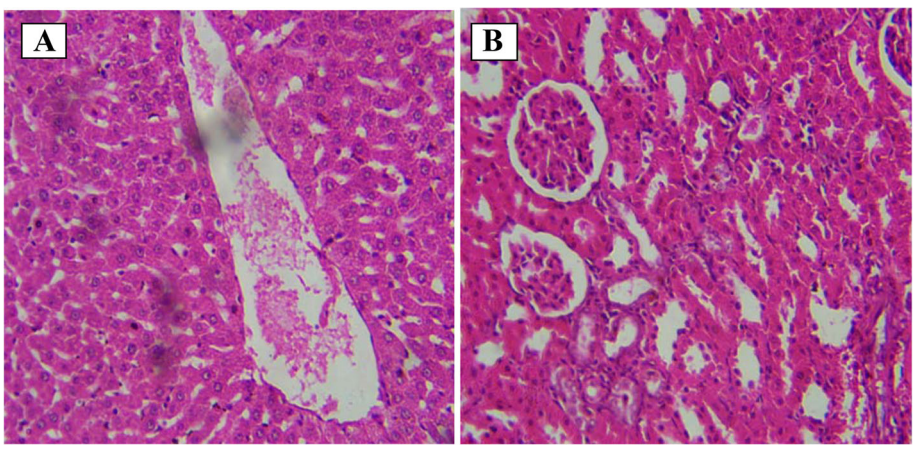

Fig. 2 Photomicrographs of liver and kidney of rat administered 5 mg/kg cisplatin (× 100, H & E stain); Morphology of liver tissue (**a**) shows damaged hepatocytes, sinusoidal dilatation, hemorrhage in portal triad, vascular congestion, infiltration of portal triad and stroma of liver by inflammatory cells, and foci of coagulative necrosis. The kidney tissue (**b**) shows congestion of interstitial spaces with presence of casts, capsular dilation, shrunken and distorted glomeruli

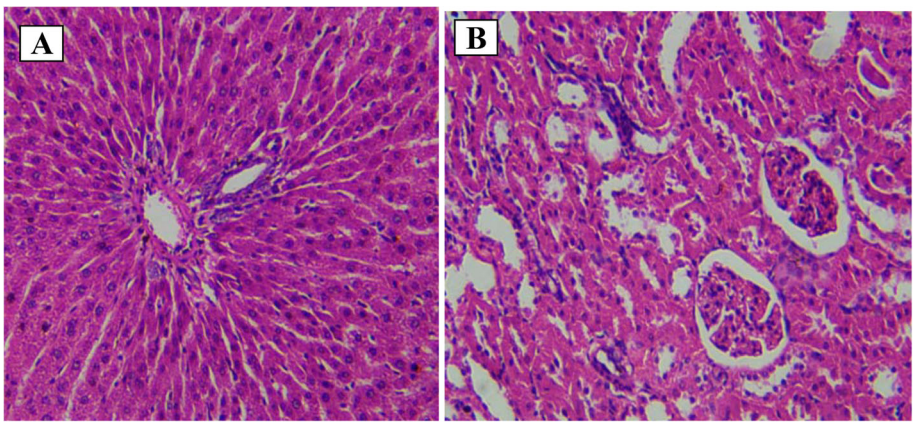

Fig. 3 Photomicrographs of liver and kidney of rat treated with 200 mg/kg *L. lanceolata* extract (× 100, H & E stain); Morphology of liver tissue (**a**) shows infiltration of hepatic cords by few inflammatory cells and small foci of coagulative necrosis. The kidney tissue (**b**) shows mild congestion of interstitial spaces, shrunken glomeruli and foci of interstitial nephritis

(TC), triglycerides (TG) and LDL-cholesterol after exposure of rats to cisplatin may be attributed to the adverse effect of this drug, leading to hepatocellular dysfunction and impaired lipid metabolism, which are in agreement with the findings of Akindele et al. [23]. It has been shown that elevated levels of TC, LDL-C and reduced level of HDL-C are risk factors for cardiovascular diseases [24]. The liver synthesizes triglycerides and incorporates them into very low density lipoprotein (VLDL) cholesterol for transport into peripheral tissues, thus when there is injury to the liver its capacity to synthesize VLDL-C is impaired, leading to elevated levels of TG [25].

Atherogenic dyslipidemia, characterized by high LDL-C/HDL-C ratio and elevated TG, is associated with high cardiovascular risk [26]. Several clinical studies tried to find a better marker of atherogenic dyslipidemia, which can predict the risk of cardiovascular diseases (CVD) and useful for assessing response to treatment instead of the classical ratio [26, 27]. It was then found that atherogenic index of plasma (AIP), as a marker of lipoprotein particle size, has predictive value for CVD risk than individual lipids and/or TC/HDL-C ratio [26, 28]. Thus, current scientific evidence indicates that AIP is a strong marker which can predict the risk of atherosclerosis and CVD [26]. The marked increase in the values of cardiac risk ratio (CRR), atherogenic coefficient (AC), and classical ratio (CR) but minimal increase in AIP of cisplatin-exposed rats may indicate a moderate risk of atherosclerosis and CVD. These findings are in agreement with previous studies, which showed that dyslipidemic and atherogenic indices increase with increasing risk of CVD and vice versa [26–29].

The significant reduction in the levels of TC, LDL-C and TG by treatment of cisplatin-exposed rats with 200

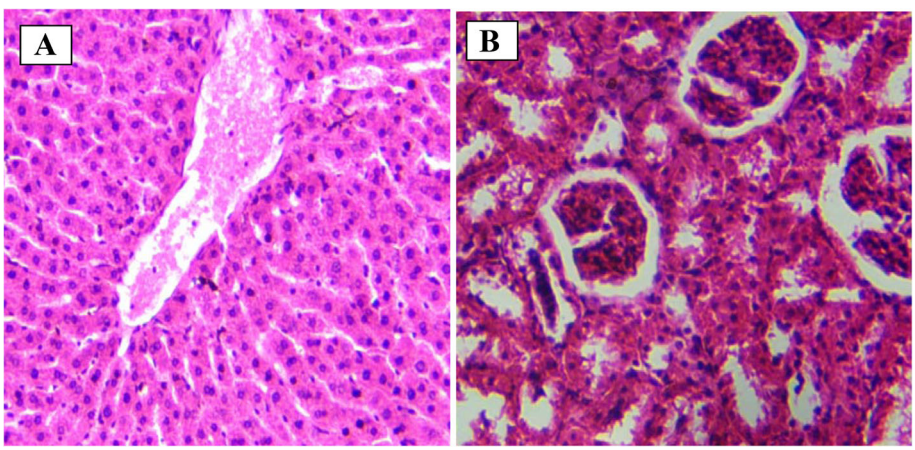

Fig. 4 Photomicrographs of liver and kidney of rat treated with 400 mg/kg *L. lanceolata* extract (× 100, H & E stain); Morphology of liver tissue (**a**) shows infiltration of portal triad and hepatic cords by inflammatory cells, and vascular congestion. The kidney tissue (**b**) shows congestion of interstitial spaces, shrunken and distorted glomeruli

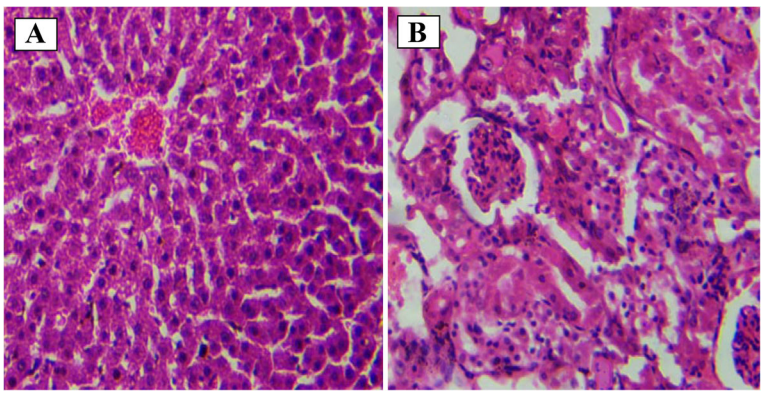

Fig. 5 Photomicrograph of liver and kidney of rat treated with 200 mg/kg *L. lanceolata* leaf extract and 5 mg/kg Cisplatin (× 100, H & E stain); Morphology of liver tissue (**a**) shows hemorrhage in the portal triad, few inflammatory cells and pyknotic hepatocytes. The kidney tissue (**b**) shows shrunken glomeruli, congestion of interstitial spaces and infiltration by inflammatory cells, showing signs of mild interstitial nephritis

and 400 mg/kg LLE may indicate that the plant extract has protective effect against cisplatin-induced hyperlipidemia in rats, when given at the appropriate dose. These findings are in agreement with Akindele et al. [23] who found that plant extract has ameliorative effect on ethanol-induced elevated levels of cholesterol, TG and LDL-C in rats. The decrease in the values of CRR, AC, AIP and CR by treatment of cisplatin-exposed rats with 200 and 400 mg/kg LLE may suggest a reduction in the risk of atherosclerosis and CVD, though the effect of low dose of extract was greater than the high dose. These findings are in agreement with Akindele et al. [23] who found that the values of CRR, AC and AIP were considerably reduced in ethanol-exposed rats by treatment with a medicinal plant extract.

The weight of animal's organ in relation to body weight is used to assess the level of tissue damage in animals [30]. It is widely accepted that alteration in the organ to body weight ratio of diseased animal is a useful indicator of tissue damage [31]. The significant reduction in the values of liver and kidney to body weight ratios after exposure of rats to cisplatin may indicate tissues degeneration and necrosis, attributed to the adverse effects of this drug. These findings are in agreement with Adebayo et al. [30] who found significant reduction in kidney to body weight ratio after exposure of rats to a toxin, and Olaniyan et al. [31] who reported that the alteration in organ to body weight ratio may be as a result of organ damage.

The significant increase in the values of liver and kidney to body weight ratios by treatment of cisplatin-

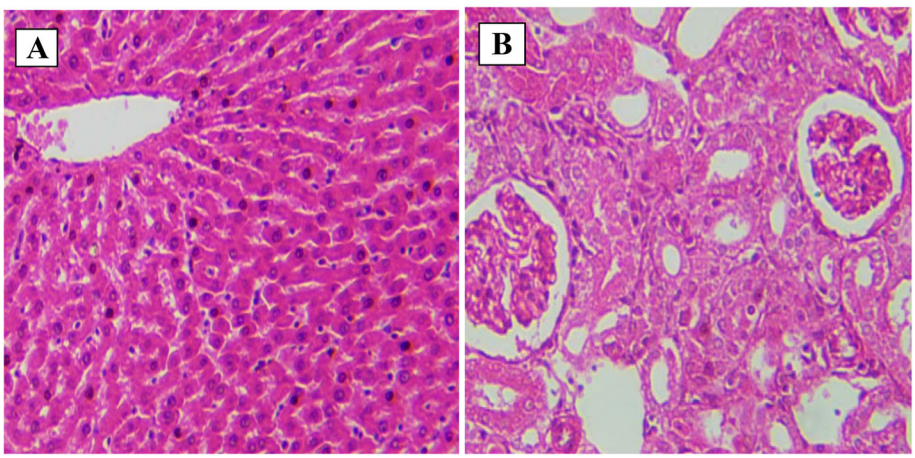

Fig. 6 Photomicrographs of liver and kidney of rat treated with 400 mg/kg *L. lanceolata* leaf extract and 5 mg/kg Cisplatin (× 100, H & E stain)**.** Morphology of liver tissue (**a**) shows mild congestion of the portal triad and infiltration of liver sinusoids by inflammatory cells and several pyknotic hepatocytes; The kidney tissue (**b**) shows shrunken and distorted glomeruli, mild hemorrhage and congestion in the interstitial spaces, showing signs of mild interstitial nephritis

exposed rats with 200 and 400 mg/kg LLE, may suggest that this plant extract has protective effect against cisplatin-induced liver and kidney injuries in rats. These findings are in agreement with Adejor et al. [32] who found that the protective effect of plant extract prevented significant alteration in organ to body weight ratio of animals. Thus, the prevention of marked decrease in liver and kidney to body weight ratios in the current study, may suggest that LLE protected the liver and kidney of rats against cisplatin-induced injuries and necrosis.

The liver and kidney photomicrographs are commonly used to evaluate the extent of tissue damage, by the microscopic study of their structural architectures. Thus, histopathological findings in tissue sections of animals are commonly used to validate the biochemical findings of a study [33]. The histopathological changes observed in the photomicrographs of cisplatin-exposed rats agreed with the biochemical findings of this study. The presence of inflammatory cells and alterations in structural architectures of liver and kidney tissues of cisplatin-exposed rats are signs of pathology; indicating degenerative changes in hepatorenal tissues, by apoptotic and necrotic processes leading to cell death. These findings are in agreement with previous studies by Alqasoumi [19] and Al-Attar et al. [33] who found pathological changes in liver and kidney structures after exposure of animals to drugs; with features such as sinusoidal dilation, parenchyma inflammation and vascular congestion in liver, glomerular and tubular modifications in nephrons. Apoptotic and necrotic cell death are important in the pathogenesis of acute liver damage [34]. Cisplatin-induced tissue toxicity may generate reactive oxygen species (ROS), leading to induction of hepatocyte apoptosis, the activation of inflammatory cells and their infiltration of tissues, as well as tissue degenerative changes [35].

There are two major molecular pathways of caspase-dependent apoptosis which are intrinsic and extrinsic [36], however the authors propose that the mechanism of cell death in this study may be by the intrinsic pathway. This pathway is activated in response to DNA damage, oxidative stress and other stress-inducing conditions. The multiple forms of stress converge on the mitochondria and cause mitochondrial outer membrane permeabilization (MOMP), which leads to dissipation of the mitochondrial membrane potential and cessation of ATP production, as well as release of a number of proteins that contribute to caspase activation [36]. In other words, intracellular stress induces the release of proteins (Bcl-2, Bak, Bax, tBid etc), which promote MOMP and release a number of proteins including cytochrome c (CYTC). The release of CYTC from the mitochondria leads to formation of apoptosome and activation of caspase 9. Then caspases 8 and 9 activate downstream caspases like the caspase 3 resulting in apoptotic cell death. The apoptotic bodies once released are rapidly recognized by neighboring cells or professional phagocytes and are generally disposed without induction of inflammation [36, 37].

The marked decrease in number of inflammatory cells, reduced degenerative changes in kidney and liver tissues, by treatment of cisplatin-exposed rats with LLE, supported the biochemical findings that this plant extract may have protective effect against cisplatin-induced hepatic and renal injuries in rats. Previous studies have shown that plant extracts could prevent pathological changes in liver and kidney tissues of animals and sometimes may reverse pathological changes already induced by drugs that are toxic to animal tissues [19, 32, 33]. Though, no functional data was determined to investigate the mechanism of protection against drug-induced tissue damage, the protective effect of LLE in the current study may be attributed to the presence of phytochemicals which can scavenge ROS and free radicals generated by the drug, thereby preventing the oxidative damage of hepatorenal tissues in rats [23]. The changes in structural architectures of liver and kidney tissues of rats after treatment with LLE only, may suggest that this plant extract may have adverse effect on animal tissues, when given at higher doses and for long duration. These findings are in agreement with Etuk and Muhammad [11] who found signs of injuries in tissues of male rats after their treatment with similar plant extract.

Conclusion

The findings of this study have shown that aqueous extract of *L. lanceolata* leaf may have protective effect against cisplatin-induced hepatorenal injuries and dyslipidemia in rats, which may be attributed to the phytochemicals present in it. This gives credence to the use of this plant extract in traditional medicine for the treatment of diseases in humans. Further studies may be conducted in order to isolate and characterize the bioactive compounds responsible for the pharmacological activities of this plant extract, and to investigate its mechanism of protection against drug-induced tissue damage in animals.

Abbreviations

AC: Atherogenic coefficient; AIP: Atherogenic index of plasma; B. wt.: Body weight; CIS: Cisplatin; CR: Classical ratio; CRR: Cardiac risk ratio; CVD: Cardiovascular diseases; CYTC: Cytochrome c; GFR: Glomerular filtration rate; LLE: *Lophira lanceolata* leaf extract; LSD: Least significant difference; Mg/kg: Milligramme per kilogramme; MOMP: Mitochondrial outer membrane permeabilization; NSAID: Non steroidal anti-inflammatory drug; ROS: Reactive oxygen species; SEM: Standard error of mean

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Authors' contributions

AHA and RJO designed the experiment and supervised it. SPA and RJO conducted the biochemical, histological and statistical analyses, and

interpreted the results. RJO wrote the first draft of this manuscript while AHA and SPA revised the manuscript for intellectual content. All authors read and approved the final draft of the manuscript.

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Availability of data and materials
The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The protocols of this study were approved by the Animal Welfare and Ethics Committee of the College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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